Improved High Soy Shrimp Feeds for the Pacific White Shrimp *Litopenaeus vannamei*

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Abstract

It could be suggested that, from a nutritional standpoint, one of the reasons for the popularity of Pacific white shrimp, *Litopenaeus vannamei*is the adaptability to a range of diets, tolerance of plant based feed ingredients and ability to utilize natural productivity. This species is very tolerant of alternative feed formulations and diets without fishmeal have been successfully implemented at the commercial level for a number of years. Despite the preponderance of information on plant based/high soy feed formulations, the wide spread acceptance of reduced fish meal feeds by the industry is still lacking. This review summarizes work we have accomplished with respect to low fishmeal or plant based/high soy feed formulations including the evaluation of new soy varieties, the assessment of SBM produced from alternative processing methods, incorporation of alternative protein

Key words: *Litopenaeus vannamei*, soybean meal, growth, digestibility, enzyme supplementation

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1. Introduction

The United States is one of the largest seafood markets in the world and shrimp is one of the most popular and valuable seafood products (Li *et al.*, 2014). Pacific white shrimp, *Litopenaeus vannamei,* is regarded as the most important cultured shrimp species worldwide and a top aquaculture commodity with a production of 3,314,447 t in 2013 (Bondad-Reantaso *et al.*, 2012, FAO, 2014). As shrimp consumption is expected to continue to increase, it is vital to develop sustainable alternative ingredients in the shrimp diets to support the rapid expansion of the shrimp industry (Achupallas *et al.*, In press).

Soybean meal (SBM) is often regarded as a cost-effective and nutritionally valuable protein source in shrimp and fish feeds. The popularity of SBM as a protein source is the result of a well-balanced nutrient profile, high digestibility, steady supply, expandable production and reasonable price (Amaya *et al.*, 2007, Davis & Arnold, 2000, Gatlin *et al.*, 2007, Lim & Dominy, 1990, Hardy, 2010, Samocha *et al.*, 2004). Although SBM is a proven alternative feed ingredient for shrimp feeds, it has a number of disadvantages including lower levels of some essential amino acid (e.g., methionine), the presence of several anti-nutritional factors (e.g. saponins, isoflavones, phytic acid, and trypsin inhibitor (Anderson & Wolf, 1995, Rackis, 1974, Rumsey *et al.*, 1994), and indigestible oligosaccharides (e.g. raffinose and stachyose) which may reduce performance of aquatic animals (Gatlin *et al.*, 2007, Knudsen, 1997, Parsons *et al.*, 2000).

Numerous studies have been conducted utilizing the soybean meal as a protein sources in aquatic animals. In a recent review, Sookying *et al.* (2013) summarized constraints when considering the move towards plant-based diets for *L. vannamei* with regard to balanced feed formulations. They concluded that the use of alternative feed formulations for *L. vannamei* is appropriate and warranted for commercial production. Over the years, many studies utilizing high soy shrimp feeds have been conducted by our laboratory at the E. W. Shell Fisheries Research Station (EWS), Auburn, AL, USA and the Claude Peteet Mariculture Center in Gulf Shores, AL, USA. A primary objective of these trials was to evaluate feed formulations that might overcome the disadvantages of conventional SBM. This review summarizes work we have accomplished in this respect

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including the evaluation of new soy varieties, the assessment of SBM produced from alternative processing methods, and the use of enzymes to improve digestibility.

2. New varieties/sources soybean meal used in diets of *L. vannamei*

Conventional SBM is available worldwide and is the dominant plant based protein source for aquatic feeds. However, as previously discussed, it generally has a moderate level of protein, relatively low levels of some essential nutrients and contains anti-nutritional factors (Herkelman *et al.*, 1992). To improve the nutritional quality of SBM one can chose to start by improving the characteristics of the soybean itself. As there is a rich history of genetic selection to improve agriculture characteristics or yield at the farm the same concepts can be applied to nutrient profiles of the soybean with the goal of producing a product of higher nutritional value.

Within our laboratory, a range of new non-genetically modified (NGM) soybean cultivars has been tested with the intent of improving SBM quality. Zhou *et al.* (2014) evaluated eight sources of SBM including six new NGM meals in practical diets formulated for *L. vannamei*. These new soy products contained higher levels of amino acids and protein as well as lower levels of some anti-nutritional factors. Results revealed significant difference in the final weight of shrimp reared on the various meals. Hence, some new lines of NGM soybean cultivars were promising and lead to improvement in the nutritional content of SBM and are suitable for inclusion in shrimp feed formulations. One of the primary advantages of these meals is an increase in protein content with meals reaching as high as 56% protein. Fang (2013) assessed thirteen soybean meal variants, which included one conventional soybean meal (FF), two commercially available NGM meals (Trifecta and 3010) and eight potential NGM varieties of which one was processed three ways (ingredients 13 processed as a meal, 14 boiled and processed as a meal, and 15 boiled and processed as press cake), in practical feed formulations for *L. vannamei*. Select meals were evaluated in an 8-week growth trial which was conducted using six replicate tanks per dietary treatment (10 shrimp per tank, initial weight 0.52 ± 0.04 g). The SBM-based reference diet was formulated using commercial SBM (45.3% diet), which was then completely replaced on an iso-nitrogenous basis with other experimental SBMs. As in the previous work, significant differences were observed in growth performance but not survival.

Fang (2013) also used principal component analysis to determine groupings of the meals based on biochemical composition. This was followed by the use of Pearson's correlation coefficients comparing growth performance of the shrimp and chemical content of the ingredients. A clear negative effect of a trypsin inhibitor on the shrimp's growth and weak correlations with stachyose levels and shrimp growth was observed. Both studies confirm that significant differences in growth are observed across a range of meals and that the level of trypsin inhibitor and possibly poorly digested carbohydrates in the meals are possible indicators of the growth performance of shrimp fed these meals.

3. Digestibility trials of different soybean sources

Digestibility data for feed ingredients is important to provide more precise information to balance feed formulations in shrimp feeds (Smith *et al.*, 2007, Zhou *et al.*, 2014). Determining digestibility of feed ingredients is widely used as an indirect assessment of nutrient availability in aquatic animals (NRC, 2011, Glencross *et al.*, 2007).

Within our laboratory, several studies of nutrient digestibility using different sources/varieties of soybean meal in *L. vannamei* have been documented. Many of these studies have utilized an equivalent reference diet and the same protocol for collection of feces and determination of digestibility coefficients (Zhou *et al.*, 2014, Fang, 2013). The apparent digestibility data for the reference diet and different soybean sources are presented in Table 1 and 2, respectively. Digestibility data for shrimp can often be quite variable compared to other aquatic species. To exemplify this we pulled data from several digestibility trials conducted over the years for which a similar basal diet was utilized. It is interesting to note that coefficients of variation across the data set (e.g. all individual data) ranged from 4.86 to 6.05% indicating globally there is about a 5% error associated with

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digestibility coefficients. Whereas the average coefficient of variation within the data sets (e.g. the average of the means for each set) was lower (2.0 to 2.9%). Clearly, there are differences between sets which are likely due to ingredient variability. However, it is our opinion this variability could also be due to differences between groups of experimental animals utilized in the trials. Overall, this indicates that the determination of digestibility coefficients within a set is quite comparable but between sets there is considerable variation and caution must be used when interpreting the data.

Table 1. Summary (mean and coefficient of variation in parenthesis) of apparent dry matter digestibility (ADMD), apparent protein digestibility (ADP), and apparent energy digestibility (ADE) of the basal diet used across a range of studies in our laboratory.

Reference	Replicates (n)	ADMD	AED	APD
1. Fang (2013)	3	68.24	74.52	85.74
		(2.47)	(2.20)	(5.02)
2. Fang (2013)	9	73.71	80.50	92.11
		(4.25)	(2.47)	(0.95)
3. Rhodes *et al.* (In press)	3	73.15	78.07	89.08
		(0.67)	(0.80)	(3.89)
4. Unpublished data	3	66.71	69.56	77.47
		(4.05)	(4.28)	(4.40)
5. Zhou *et al.* (2014)	4	72.25	77.92	88.95
		(0.84)	(0.71)	(1.09)
Average of mean CV	5	2.33	2.07	2.91
Average of all the data	22	71.67	77.39	88.26
CV		4.86	5.31	6.05

Coefficient of Variation (CV)

Table 2. Apparent dry matter digestibility (ADMD), apparent protein digestibility (ADP), and apparent energy digestibility (ADE) of individual ingredients determined with juvenile shrimp using the reference diet technique (70:30 ratio).

	ADMD	ADP	ADE
Zhou *et al.* (2014)			
RD-A	85.2±4.9^{ab}	93.8±3.9^{b}	90.4±3.0^{a}
RD-B	71.3±5.3^{c}	96.9±4.6^{ab}	76.6±4.6^{b}
RD-C	88.3±2.2^{a}	99.8±1.3^{a}	90.6±1.1^{a}
RD-D	75.4±7.6^{bc}	94.2±2.3^{b}	83.2±6.4^{ab}
RD-E	76.8±13.6^{abc}	93.6±3.9^{b}	82.2±12.3^{ab}
RD-F	80.9±7.6^{abc}	95.7±2.1^{ab}	85.4±6.5^{ab}
RD-G	75.3±6.4^{bc}	95.6±1.3^{ab}	81.4±5.4^{ab}
RD-H	85.5±6.6^{ab}	97.7±1.9^{ab}	91.3±5.4^{a}
PSE[2]	1.31	0.52	1.12
P-value	0.0350	0.0750	0.0300
Fang (2013)			
Basal I	73.4±3.66^{bc}	80.2±1.39^{ab}	89.2±3.58 ^{a}
I-10	70.3±1.91^{bc}	76.2±4.38^{b}	91.9±1.27 ^{a}
I-11	77.9±2.17^{bc}	84.3±1.97^{ab}	96.3±1.67 ^{a}
I-12	63.4±5.43^{c}	72.5±4.46^{b}	90.4 ±3.34 ^{a}
I-13	64.8±4.16^{c}	73.0±2.28^{b}	80.3±2.13 ^{b}
I-14	76.6±7.69^{bc}	84.4±5.72^{ab}	83.1±5.40 ^{b}
I-15	78.0±2.02^{bc}	81.6±2.74^{ab}	93.7±1.58 ^{a}
I-16	78.5±13.74^{bc}	81.4±11.58^{ab}	92.7±1.88 ^{a}
I-17	82.1±11.69^{bc}	86.3±11.20^{ab}	96.3±3.59 ^{a}
I-18	95.4±4.43^{a}	96.1±2.63^{a}	98.0±0.69 ^{a}
I-19	73.4±3.66^{bc}	80.2±1.39^{ab}	89.2±3.58 ^{a}
Basal II			
FF	79.8±5.38^{bc}	83.0±3.96^{ab}	95.1±2.94^{a*}
Trifecta	77.8±6.27^{bc}	83.3±6.64^{ab}	90.4±3.56^{a}
3010	86.5±2.69^{ab}	88.7±2.27^{ab}	91.7±4.79^{a*}
PSE	1.81	1.58	0.84

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P-value	0.0003	0.0025	0.0001

[1] Mean of four replicates. Base on Duncan test, Number within the same column with different superscript are significant different ($P < 0.05$). [2] Pooled Standard Error.

Zhou *et al.* (2014) assessed eight sources of soybean meal that include six new NGM soy varieties in *L. vannamei* feed formulations. In that study, to elucidate the interaction between apparent digestibility data and chemical characteristics of test ingredients, correction analysis followed by regression analysis was employed. Results from that study confirmed that protein dispersion index (PDI) and some antinutrient levels were decreased with increased processing temperature. These results are in agreement with previous studies by Francis *et al.* (2001), Genovese *et al.* (2006), and (Qin *et al.*, 1996). Additionally some ingredients with high levels of trypsin inhibition had reduced apparent digestibility coefficients (ADP and ADE) confirming the effect of a trypsin inhibitor.

In another study by Fang (2013), thirteen ingredients (as previously described) were assessed in feed formulations for *L. vannamei* using principle component analysis (PCA) to evaluate a wide range of variable and possible effects from multiple factors of chemical characteristics and processing. Results indicated that some ingredients had common characteristics while others were markedly different. Thus, different biological responses could results. Both studies indicated that some new lines of soybean meal could improve the digestibility coefficients in shrimp diets.

4. Validation in outdoor ponds and tank based systems.

There are numerous aquaria or clear water laboratory studies that have evaluated the use of soybean meals in shrimp feeds. However, until validated under commercial conditions, farmers are hesitant to pursue alternative diet formulations. Once alternative soybean meals are validated in indoor clear water systems where natural foods are not present the results can be validated outdoor tanks and research ponds. Within our laboratory, numerous pond based trials with parallel outdoor door tank studies have been conducted (list in Table 3 and 4, respectively). These studies, as well as those conducted by other laboratories, have demonstrated that *L. vannamei* can accept a wide range of alternative feed formulations culturing under a variety of research condition (Achupallas *et al.*, In press, Sookying & Davis, 2011, Sookying & Davis, 2012, Yu *et al.*, 2013, Roy *et al.*, 2009).

Table 3. Pond production trials of *Litopenaeus vannamei* fed experimental diets containing high levels soybean meal cultured in 0.1-ha ponds at Claude Peteet Mariculture Center in Gulf Shores, Alabama, USA.

Treatment	% inclusion SBM	SD (shrimp/m^2)	Period (wk)	IBW (g)	FBW (g)	Yield (kg/ha)	FCR	Survival (%)	Reference
9% FM	32.48	35	18	0.030	19.6	5847	1.24	87.2	Amaya *et al.* 2007
6% FM	34.82				18.4	5363	1.38	84.0	
3% FM	37.17				19.8	6548	1.12	94.0	
0% FM	39.52				20.7	6347	1.14	87.4	
10% PBM	55.12	35	18	0.038	16.0	5187	1.33	93.7	Sookying and Davis 2011
10% FM	53.71				16.9	5054	1.35	86.6	
10% DDGS	58.01				16.3	5265	1.32	92.2	
10% PM	58.00				16.2	5194	1.37	88.6	
17shrimp/m^2	53.24	17	16	0.015	25.3	2660	1.17	61.5	Sookying *et al*. 2011
26shrimp/m^2	53.24	26			20.7	3052	1.50	58.0	
35shrimp/m^2	53.24	35			22.0	4612	1.54	59.5	
45shrimp/m^2	53.24	45			21.9	6149	1.35	65.1	
0% SPC	58.01	35	18	0.013	13.5	4190	1.54	86.7	Sookying and Davis 2012
4% SPC	52.01				15.7	5051	1.28	89.5	
8% SPC	46.01				13.5	4508	1.45	92.9	
12% SPC	39.67				13.5	4479	1.44	93.3	
Fish oil	53.24	38	17	0.034	18.9	5254	1.40	74.0	Silva 2013
Soybean oil	53.24				18.0	5141	1.43	75.4	
Poultry grease	53.24				21.6	5070	1.45	65.6	
Flaxseed oil	53.24				21.0	5363	1.37	68.2	
0% CPC	46.69	38	16	0.023	20.5	5007	1.38	64.9	Yu *et al.* 2013
4% CPC	46.63				17.5	5190	1.34	77.6	

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8% CPC	46.48				17.2	5420	1.27	83.6	
12% CPC	46.32				18.2	5440	1.29	75.9	
0% GDDY	53.56	30	16	0.038	21.4	5527	1.03	86.5	Achupallas *et al*. in press
5% GDDY	48.17				19.8	5292	1.09	89.4	
10% GDDY	42.80				23.1	4760	1.23	69.6	
15% GDDY	37.51				21.7	5606	1.02	86.9	

Stocking density (SD), Initial body weight (IBW), Final body weight (FBW), Fish meal (FM), Poultry by product meal (PBM), Distillers dried grains with solubles (DDGS), Pea meal (PM), Soy protein concentrate (SPC), Grain distillers dried yeast (GDDY).

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Table 4. Outdoor tank production trials (800 L/tank) of *Litopenaeus vannamei* fed experimental diets containing high levels soybean meal cultured in semi-recirculating system in Claude Peteet Mariculture Center in Gulf Shores, Alabama, USA.

Trt	% inclusion SBM	SD shrimp/m^2)	Period (wk)	IBW (g)	FBW (g)	Biomass (g/tank)	FCR	Survival (%)	Reference
9% FM	32.48	37.5	11.6	0.74	18.5	586.2	1.13	85.0	Amaya *et al.* 2007
6% FM	34.82				18.6	624.0	1.12	89.2	
3% FM	37.17				18.8	622.5	1.11	88.3	
0% FM	39.52				17.4	564.4	1.20	86.7	
10% PBM	55.12	37.5	12	2.13	18.7	553.0	1.32	98.7	Sookying and Davis 2011
10% FM	53.71				18.8	557.7	1.31	98.7	
10% DDGS	58.01				18.5	548.3	1.33	98.7	
10% PM	58.00				19.0	554.4	1.31	97.3	
15 shrimp/m^2	53.24	15	10	2.82	16.1	193.5	1.15	100.0	Sookying *et al.* 2011
25 shrimp/m^2	53.24	25			15.7	286.2	1.26	96.1	
35 shrimp/m^2	53.24	35			14.1	376.3	1.39	99.1	
45 shrimp/m^2	53.24	45			14.6	461.3	1.41	93.4	
55 shrimp/m^2	53.24	55			13.4	543.6	1.52	96.4	
65 shrimp/m^2	53.24	65			13.6	637.2	1.54	95.4	
0% SPC	58.01	37.5	10	1.00	13.5	398.9	1.28	98.0	Sookying and Davis 2012
4% SPC	52.01				13.7	397.2	1.28	96.7	
8% SPC	46.01				13.9	416.8	1.22	100.0	
12% SPC	39.67				15.0	411.5	1.23	98.7	
Fish oil	53.24	37.5	12	0.25	14.7	502.4	1.05	96.7	Silva 2013
Soybean oil	53.24				13.8	473.8	1.11	97.5	
Poultry grease	53.24				14.8	483.5	1.07	92.5	
Flaxseed oil	53.24				14.0	481.9	1.09	98.3	
0% GDDY	53.56	37.5	12	3.05	18.5	535.8	1.25	96.7	Achupallas *et al.* in press
5% GDDY	48.17				18.1	534.6	1.25	98.3	
10% GDDY	42.80				19.0	530.6	1.29	93.3	
15% GDDY	37.51				18.3	534.8	1.26	97.5	

Stocking density (SD), Initial body weight (IBW), Final body weight (FBW), Fish meal (FM), Poultry by product meal (PBM), Distillers dried grains with solubles (DDGS), Pea meal (PM), Soy protein concentrate (SPC), Grain distillers dried yeast (GDDY).

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Given that SBM is one of the primary protein sources in aquaculture feeds and the fact that it is widely available will likely increase the use of SBM in shrimp feeds (Sookying *et al.*, 2013, Lim *et al.*, 1998). Within our laboratory, the first indications that high levels of soybean meal could be used in shrimp feed was actually the result of work with a co-extruded wet poultry waste product (Davis & Arnold, 2000). This product was primarily soybean meal and thus indirectly demonstrated a high tolerance to soybean meal. The efficacy of these diets was later demonstrated in outdoor tanks (Samocha *et al.*, 2004). A range of studies identifying limiting nutrients and validating the response to nutrient limitations when natural foods were present in outdoor or green water systems have been conducted. This included evaluating the response of shrimp reared in outdoor tanks to: reduced levels of marine oils and highly unsaturated fatty acid (HUFA) supplements (Gonzalez-Felix *et al.*, 2010, Samocha *et al.*, 2011, Patnaik *et al.*, 2006, Samocha *et al.*, 2010) cholesterol supplements (Morris *et al.*, 2011); as well as phospholipids, phosphorus (see below) and various attractants such as squid liver meal (Morris, 2008). These studies confirmed that nutrient limitations induced under laboratory conditions can also be observed under field conditions when natural foods are present.

These studies complimented a series of studies to demonstrate the removal of fishmeal with poultry by-product meal (Amaya *et al.*, 2007) followed by the removal of poultry by product meal to produce a plant based feed formulation (Sookying & Davis, 2011). Over the years we have looked at a range of high soy feed formulations in combination with a number of proteins sources including fishmeal, poultry by-product meal, distillers dried grains with solubles, pea meal, soy protein concentrate, and corn gluten meal. Results of these studies are summarized in (Table 1 and 2).

Our most recent work, Achupallas *et al.* (In press), demonstrated that the high inclusions of SBM combined with different levels of grain distillers dried yeast can provide adequate growth performance in *L. vannamei* reared in outdoor tanks and production ponds. While pond based data provides a demonstration in the most widely utilized culture unit by the commercial industry, these results still needed to be validated using more statistically robust methods. Hence, these studies were all confirmed in parallel using outdoor tanks where water quality and natural foods are equalized. The validity of these trials are confirmed by excellent growth rates, adequate survival and good FCRs (Table 4). These data confirm that high soy shrimp feeds can be successfully used in diets for *L. vannamei*.

5. Enzyme supplementation – phytase

The presence of anti-nutrients in plant-based feed ingredients has been a challenge for the commercial feed mills. Thus, a number of studies have been conducted to evaluate the potential of reducing the adverse impact of anti-nutrients in plant-based feed formulations in shrimp. Phytate is one anti-nutrient found in most seeds and present in high enough levels in soybean meal to negatively impact growth performance of shrimp.

Phytate can produce a negative impact on nutrient digestibility and mineral availability (Kumar *et al.*, 2012). Fifty to eighty percent of the total phosphorus (P) content in plant seeds is found in the form of phytate (Ravindran *et al.*, 1995). Phosphorous in this form is generally not bioavailable to shrimp due to the lack of the intestinal digestive enzyme, phytase which is required to release P from the phytate molecule (Jackson *et al.*, 1996). Phosphorus is the most commonly supplemented macro-mineral which not only adds to the cost of the diet but also contributes to pollution loading of the culture system and the surrounding environment.

Phosphorus requirements of shrimp have been relatively well studied under laboratory conditions Davis *et al.* (1993). Few studies have been carried out under field conditions where natural foods could supplement the requirement. To determine if P is limiting in plant based diets under field conditions, three diets were formulated 1) without a P supplement, 2) nutritionally adequate P supplement and 3) P in excess of the requirement (Table 5).

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Table 5. Ingredient composition (g 100 g^{-1} as is) of three experimental diets designed to contain 35% protein and 6.4% lipid using primarily plant-based ingredients. The three diets included a basal diet without a P supplement (0.59 % P), a diet with P supplement designed to meet the requirement (1.07% P) and a diet in excess of the requirement (1.53 % P).

	0.59% P*	1.07% P	1.53% P
SE Soybean meal	60.5	60.5	60.63
Corn Gluten meal	8.0	8.0	8.0
Menhaden Fish Oil	6.4	6.4	6.4
Wheat starch	3.93	0.93	0.0
Whole wheat	18.0	18.0	15.8
Trace Mineral premix	0.5	0.5	0.5
Vitamin premix	1.8	1.8	1.8
Choline chloride	0.2	0.2	0.2
Stay C 250 mg/kg	0.07	0.07	0.07
CaP-diebasic	0.0	3.0	6.0
Lecithin	0.5	0.5	0.5
Cholesterol	0.1	0.1	0.1

*P is the analyzed value as determined by New Jersey Feed Laboratory.

The test diets were then offered to replicate groups of shrimp under standardized conditions. The methods used in this study were similar to those that have been used in numerous published studies from the AgriLife facility. The study was initiated with juvenile shrimp (0.59 ± 0.17 g) and conducted over an 84-day period in outdoor tanks (650 L working volume and 0.85 m^2 bottom area) stocked at 30 shrimp m^{-2}. The shrimp were feed twice daily throughout the study. The response of shrimp to plant based diets containing various levels of P supplements is summarized in Table 6. At the conclusion of the trial, average weight, growth, FCR and yield of the shrimp were significantly improved by the addition of P to meet the dietary requirement. These results confirm that even in the presence of natural productivity, shrimp reared on diets without animal meals are deficient in P. Furthermore, the addition of P in excess of the requirement does not improve growth and would simply contribute to increased costs and pollution loading. An alternative to supplementing high levels of P to plant based diets is to increase the biological availability of P in the feed.

Table 6. Mean final weight, survival, yield and FCR values of the Pacific white shrimp, *Litopenaeus vannamei*, reared for 84 days on diets with different levels of phosphorus in an outdoor tanks system. Values represent the mean of five replicates. Within a column, values with different letters are significantly different based on Student-Newman-Keuls multiple range test.

Total Phosphorus	Biomass (g)	Final Weight (g)	% Survival
0.59%	488.3[a]	18.8[a]	96.2
1.07%	515.4[b]	19.9[b]	99.2
1.53%	534.9[b]	20.6[b]	97.7
P value	0.0023	0.0008	0.5199

Phytases (myo-inositol hexakiphosphate phosphohydrolase) are a group of enzymes that can catalyze the hydrolysis of phytate, and reduce the negative effects of phytate on nutrient availability in monogastric animals (Mullaney *et al.*, 1999, Mitchell *et al.*, 1997). Several studies have documented that phytase can help improve P bioavailability, growth performance, P digestibility when feeding phytase-supplemented feeds, and the efficacy of top spraying diets with phytase in fish (Zhu *et al.*, 2014, Papatryphon *et al.*, 1999, Sajjadi & Carter, 2004, Sugiura *et al.*, 2001, Yoo *et al.*, 2005, Sugiura *et al.*, 1999, Liu *et al.*, 2012, Liebert & Portz, 2005, Cain & Garling, 1995, Vielma *et al.*, 2004, Jackson *et al.*, 1996, Eya & Lovell, 1997, Rodehutscord & Pfeffer, 1995, Vielma *et al.*, 2000, Debnath *et al.*, 2005) and shrimp (Biswas *et al.*, 2007, Fox *et al.*, 2006). A review of the application of microbial phytase in fish feed was reported by Cao *et al.* (2007). However, limited data on the use phytase in *L. vannamei* are available to date.

Qiu (2015) conducted a series of growth and digestibility trials on the response of *L. vannamei* fed a high soy based diet with graded levels of phytase supplementation. Results revealed that shrimp growth performance, P and protein retention, and P whole body content were not significantly influenced by dietary phytase supplementation in the high soy based diet. However, protein digestibility was improved by phytase inclusion at both 500 and 2000 IU/kg. P digestibility was also improved by 2000 IU/kg phytase supplementation. Based on these results, we demonstrated that the phytase supplementation can improve the P and protein digestibility in *L. vannamei*.

With the exception of phytase, other enzyme supplements are not widely utilized and/or there is limited data. There are several proteinases and carbohydrates, which could be added to aquatic animal feeds when high inclusions levels of alternative ingredients are utilized (Hardy, 2000). Hence, more studies for other enzyme supplements should be pursued.

6. Conclusions

It is abundantly clear that a high soy shrimp diet is accepted by Pacific white shrimp as long as the diets have balanced levels of nutrients. Imbalances in nutrition, the use of poor quality ingredients and or sensitivity to some components of soybean meal can lead to poor performance. However, given the cost and benefits of soy based feed formulations the industry is strongly encouraged to reduce animal protein and in particular fish meal, to levels that provide the best economic returns.

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